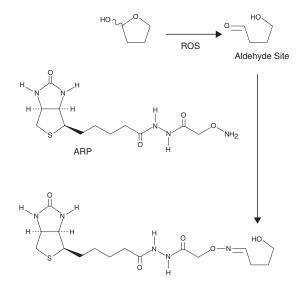


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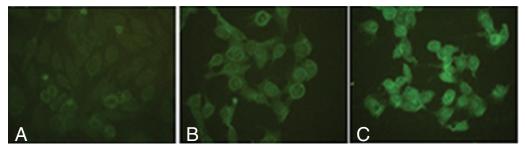
Aldehyde Site (DNA & Protein) Detection Kit

Oxidative damage occurs in all living organisms from reactive oxygen species (ROS), which are a consequence of normal body processes such as metabolism. ROS react with proteins, resulting in protein modification, such as introduction of carbonyl groups. The modified proteins are dysfunctional and can be removed through degradation. Both mitochondrial DNA and nuclear DNA are constantly exposed to oxygen radicals, causing extensive oxidative damage. DNA damage by ROS has significant consequences since it causes mutations and genomic instability. Studies have shown that oxidative DNA damage accumulates with aging and has been implicated in many diseases, including cancer.¹ Assessment of this damage in various biological matrices is essential for understanding the mechanisms of oxidative damage and its biological effects.

Over the past decades, analytical techniques such as LC- and GC-mass spectrometry, have been developed to measure oxidative DNA base damage. More recently, antibody-based immunoassays, assays involving the use of DNA repair glycosylases, such as the comet assay, and slot blot assays using an aldehyde reactive probe (ARP), such as O-(biotinylcarbazoylmethyl) hydroxylamine, have been used to assess oxidative DNA damage.^{2,3} The ARP probe reacts specifically with aldehyde groups that result from protein or DNA modification. By using an excess amount of ARP, aldehyde sites in both protein and DNA can be converted to biotin-tagged aldehyde sites, which can be detected using avidin-conjugated reporters.



Cayman's Aldehyde Site (DNA and Protein) Detection Kit employs an ARP, O-(biotinylcarbazoylmethyl) hydroxylamine, as a probe to detect aldehyde sites in cells. Epigallocatechin Gallate (EGCG), a compound known to react with culture medium to generate hydrogen peroxide (H_2O_2) and cause DNA damage, is included as a positive control. The kit is easy to use and can be easily adapted to high throughput screening for compounds imposing oxidative stress on cells or organisms.



Hydrogen peroxide induces oxidative damage in HeLa cells. HeLa cells were plated at a density of 1 x 10⁴ cells', well in a 96-well plate. The next day, cells were treated with vehicle (control), 0.015% (4.9 mN) H₂O₂, or 0.03% (9.8 mN) H₂O₂ for four hours. Cells were then processed for staining. Panel A: cells treated with vehicle (control) had background levels of oxidative protein/DNA damage, appearing as faint staining both in the cytoplasm and nuclei. Panel B: cells treated with 0.015% (4.9 mN) H₂O₂ had elevated levels of oxidative damage in both cytoplasm and nucleus, as evidenced by an increase in staining intersity. Panel C: cells treated with 0.03% (9.8 mN) H₂O₂ had strong staining, indicating severe oxidative damage.

96 wells

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References

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